THE GROWTH OF THE NUCLEUS IN THE DEVELOPING EGG OF CHLOROHYDRA VIRIDISSIMI

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Introduction and Historical

The development of the egg of *Hydra* has been studied quite thoroughly by many workers, chief of whom are Kleinenberg (1872), Brauer (1891), Downing (1908), Tannreuther (1908), Wager (1909), and Kepner and Looper (1926). All of these workers have pointed out more or less definitely that the eggs arise from indifferent cells, that the developing egg grows at the expense of the surrounding modified interstitial cells which are eventually taken into the egg itself, and that apparently only one nucleus survives and becomes the nucleus of the egg. It is interesting to note in passing that Kepner and Looper (1926) were the first to show that the earlier stages of the development of the egg are more dependent upon the endoderm for their substance than upon the modified interstitial cells of the ectoderm surrounding them. This fact had apparently been overlooked by other workers along this line. Most workers have come to the conclusion that the egg arises from an apparently unmodified, interstitial cell of rather average size, which in its earlier stages cannot be distinguished from the other interstitial cells until the process of ovary formation is very much advanced. Several workers have observed that from one to several eggs may start simultaneous development in an ovary. Usually, however, only one survives and grows to maturity. Downing (1909), on the other hand, found that an ovary of *H. diœcia* may produce several eggs, and further, advances the theory that the interstitial cells which will later develop into eggs are always distinguishable from the other interstitial cells by their size, peculiar nucleus and cytoplasmic inclusion adjacent to the nucleus, and that they are self-propagating and thus handed down from generation to generation.

These studies and differences of opinion, however, deal mostly with the origin of the ovary, the development of the eggs by taking their substance from the endoderm during the earlier stages, and the appropriation of the surrounding modified interstitial cells during the later stages of growth; but since none of these works deal with or demonstrate, except in a minor way, the development of the nucleus of the egg, it was decided to make a study of it at its various periods of development.

Material and Methods

Chlorohydra viridissimi was used exclusively in this experiment. A well-balanced laboratory culture was made early in the month of August, and the animals became sexually mature about September first. It was observed that these *Hydra* were producing only one ovary from which apparently only one egg was being matured, so they thus lent themselves quite admirably as material for the study of the development of the nucleus. The method of studying these nuclei was as follows: The *Hydra* were removed to a slide upon which a square of vaseline had been marked out with a match stick. The *Hydra* were placed in the center of this square in as large an amount of Mundie's macerating fluid as possible and then covered with a cover-glass. By drumming very lightly on the cover-glass with a needle it was possible to work the egg out of the ovary; further slight drumming burst the egg and allowed the nucleus to be seen. By carefully sliding the cover-glass to one side or the other it was possible to roll the nucleus out and away from the majority of the surrounding pseudocells where it could be studied, measured, and photographed. Extreme caution was exercised to see that the nuclei were not brought under any pressure so that they remained in their original round condition. When these nuclei were measured, care was taken that the focusing was so adjusted that the nuclei were measured at their equator; this same precaution was exercised when they were photographed. In addition to the above, a series of measurements and observations were made upon nuclei which had been worked out in aquarium water. As far as could be ascertained no measurable shrinkage or swelling could be found, so the work was continued using the Mundie's macerating fluid since the nuclei could be preserved in their almost original condition for from 15 to 20 hours, if precautions were taken to see that the cover-glasses were well sealed with vaseline.

A compound binocular microscope, equipped with 10 ×, 43 ×, and 97 × objectives, and 5 ×, 10 ×, and 15 × oculars, was used in this study.

The Interstitial Cells

Leydig (1848) was the first worker to describe the interstitial cells of *Hydra*. These small cells lie between the epithelio-muscular cells of both the ectoderm and endoderm. They are most numerous in the ectoderm between the epithelio-muscular cells of the upper two-thirds of the *Hydra*'s body; one rarely finds undifferentiated interstitial cells on or in the tentacles; in the stalk and basal disc they are also often en-

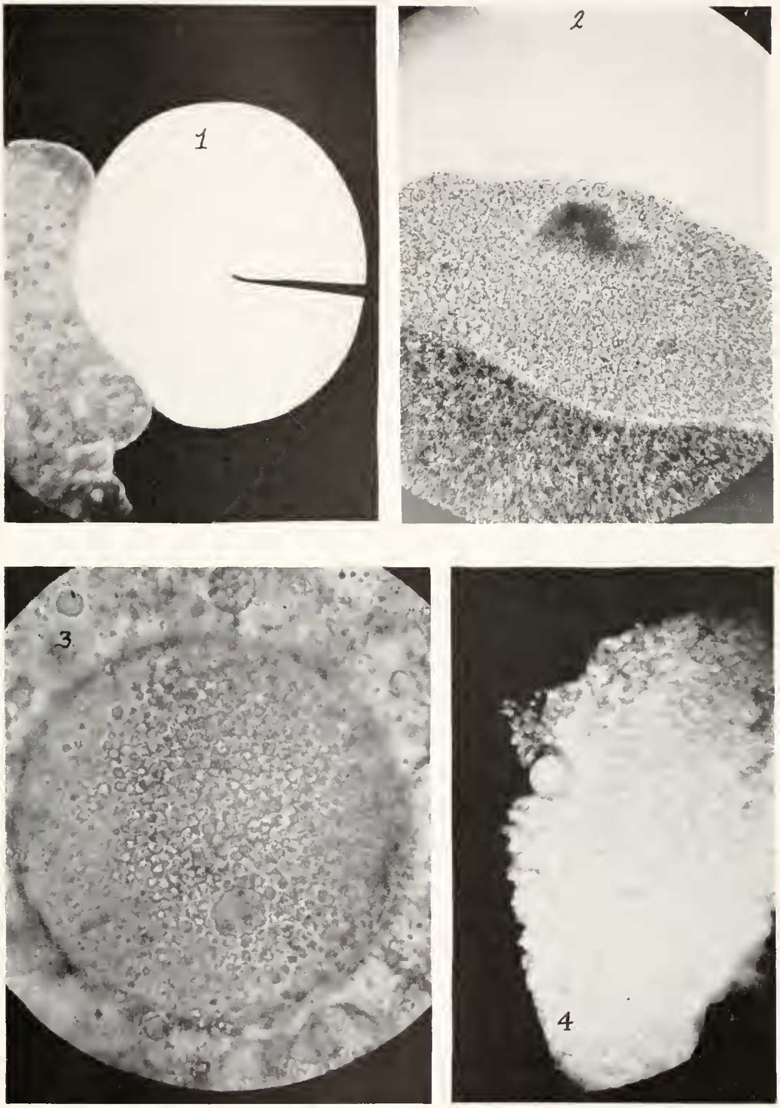

PLATE I

1. Mature egg before process of maturation has set in. Tip of pointer indicates position of nucleus. $\times 90$. Dark field illumination photograph.

2. Dark area indicates a very small egg in the ovary. $\times 90$.

3. Mature nucleus just before process of maturation has set in. $\times 776$.

4. Dark field illumination photograph of an egg crushed in Mundie's macerating fluid. Note size of nucleus as compared to the surrounding pseudocells. $\times 90$.

countered in varying numbers. Various workers have slightly different terminologies in describing these cells, such as follows:

First: Those cells which were developed or were developing into stinging cells have been called "cnidoblasts," "cnidocyts," "nematoblasts," and "nematocyts"; some have even used the word "nematocyst" to designate the interstitial cell with its nucleus, cytoplasm, and cell organelle, or coiled stinging thread.

Second: Some have called all of the cells which lie between the epithelio-muscular cells "interstitial cells" regardless of whether they were in any wise differentiated and,

Third: Some have used the words "indifferent cells" to designate those which were in no wise differentiated. We see then that some ambiguity has arisen through the various terminologies which have been employed. It is true, they are all "interstitial cells," but not all are "indifferent cells." The interstitial cells include all of the cells which lie between the epithelio-muscular cells of the body of the *Hydra* of both the endoderm and the ectoderm. The "indifferent cells," which are also interstitial, are the interstitial cells of both the endoderm and the ectoderm which are not differentiated in any way and which may be modified as needed to various specialized interstitial cells of either endoderm or ectoderm. The total number of "interstitial cells" remains almost constant in relation to the size of the animal; but the number of "indifferent cells" apparently varies greatly at different times; these cells also vary greatly in size and location; sometimes they lie down close to the bases of the epithelio-muscular cells near the mesoglea, at the same time others are found out near the inner surfaces of the epithelium; they may also be found among groups of developing cnidoblasts or in great groups alone. They are occasionally found, apparently unmodified, in developing ovaries and under testes. Their shape varies

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Plate II

1. Light field illumination photograph of an egg crushed in Mundie's macerating fluid. × 90.

2. The nucleus after maturation as it awaits fertilization at the bottom of a small depression in the egg membrane. Compare with No. 6, Plate III. × 776.

3. Nucleus of a very young egg. Note nucleolus. This was the smallest nucleus found in macerated material. Nuclear diameter 17.5 microns. × 320.

4. From macerated material. Nuclear diameter 24.5 microns. × 320.

5. Macerated material. Nuclear diameter 41.5 microns. × 320.

6. Macerated material. Nuclear diameter 43.80 microns. × 320.

7. Macerated material. Nuclear diameter 54.25 microns. × 320.

8. Macerated material. Nuclear diameter 63.5 microns. × 320.

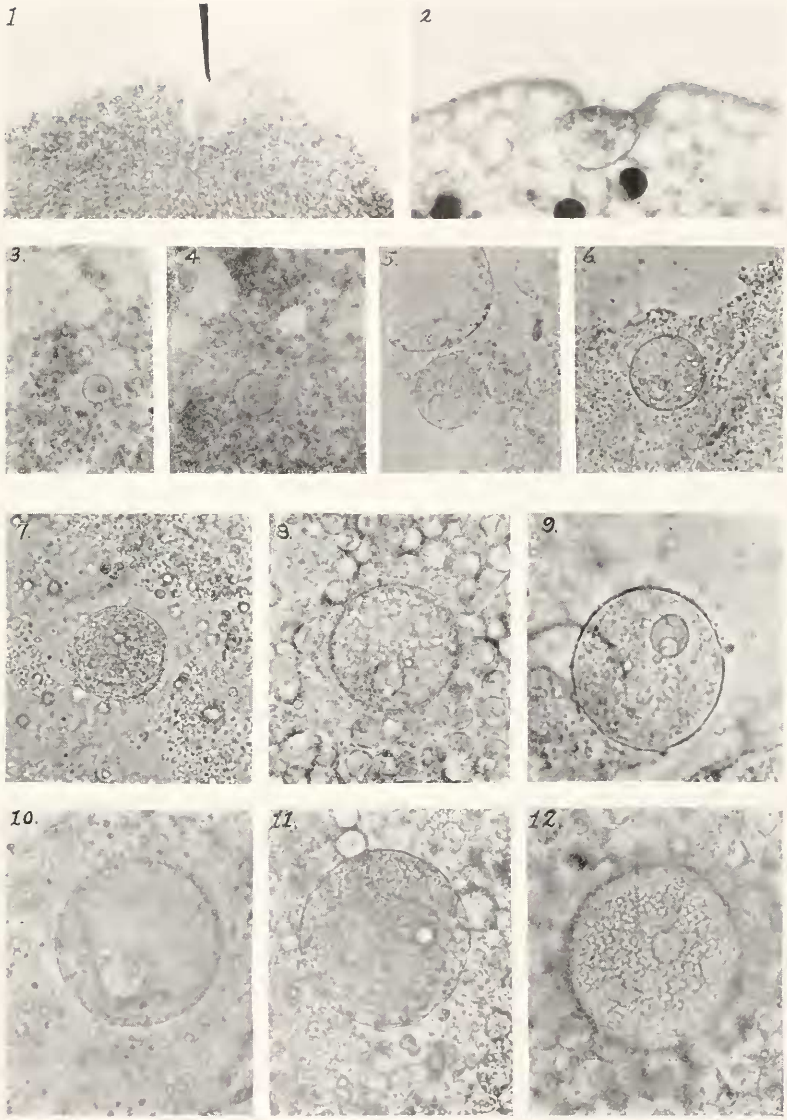
9. Macerated material. Nuclear diameter 86.75 microns. × 320.

10. Macerated material. Nuclear diameter 94.5 microns. × 320.

11. Macerated material. Nuclear diameter 96.25 microns. × 320.

12. Macerated material. Nuclear diameter 103 microns. × 320.

PLATE II



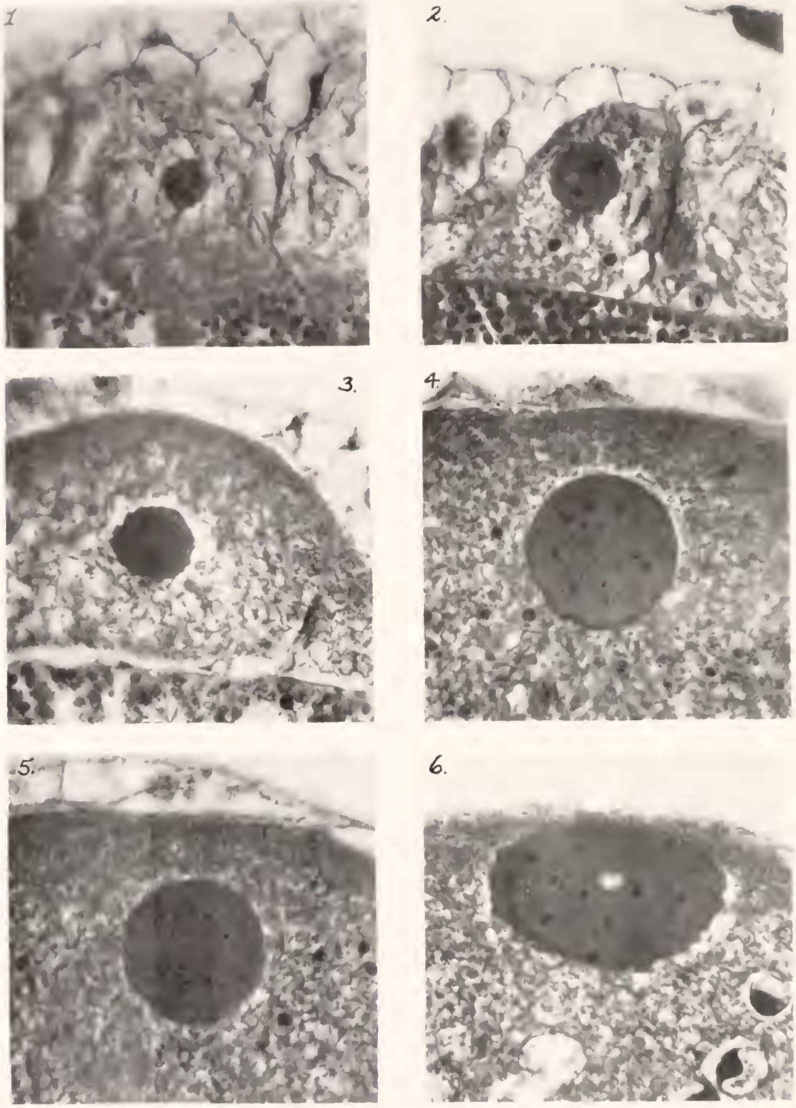


PLATE III

1. Fixed material. Nuclear diameter 9.25 microns. × 450.
2. Fixed material. Nuclear diameter 12.95 microns. × 450.
3. Fixed material. Nuclear diameter 20.50 microns. × 450.
4. Fixed material. Nuclear diameter 38.50 microns. Note how the nucleoli have been increased in 4, 5, and 6. × 450.
5. Fixed material. Nuclear diameter 37.50 microns. × 450.
6. Fixed material. Nuclear diameter 110 × 90 microns. Note that this nucleus is lying just under the egg membrane. × 450.

greatly in accordance with the number of other interstitial cells in the neighborhood and the region of the body in which they are found. The expansion and contraction of the animal's body, it has also been noted, vary the shape of the cells somewhat. During the late prophase of mitosis they assume a rounded condition. The nucleus has a very distinct membrane, and the entire nucleus takes the commoner nuclear stains quite readily and quite intensely. The number of nucleoli varies from one to three, and they vary greatly in their ability to take up various staining solutions.

The Appearance of the Egg and Its Later Development

In sectioned material the egg may be identified much earlier than in macerated material. As has already been pointed out in many works along this line, one of the primary steps of ovary formation is the increase in size of the indifferent cells in the area where the ovary is to be formed. This increase in size continues, in many cases, until these cells have enlarged from eight to ten times their original size. During the process of enlarging, their cytoplasm becomes highly vacuolated and their nuclei degenerated. In studying sections through an ovary one finds that the future eggs do not present any of the characteristics of the modified indifferent cells and take the ordinary stains quite differently from the surrounding cells which they later engulf. When the *Hydra* bearing ovaries are studied slightly compressed the eggs may be identified at an early stage by their color, position in the ovary, and different refraction of the light passing through the ovary. However, it is very difficult to identify the eggs in this manner until they are larger than the average modified indifferent cells which surround them.

Kepner and Looper (1926) have shown that the egg depends first upon the endoderm for its material. This process, as they have demonstrated, is continued until the egg is of quite large size and has developed many pseudopodia which radiate from a more or less central area in all directions between the cells of the ectoderm. Many observations, made on both sectioned and living material, have convinced me that these pseudopodia never invade a region or area in which the indifferent cells are not modified. The boundaries of the ovary are very distinct during all of the process of ovogenesis.

The nucleus grows very fast and apparently is always in proportionate size to the size of the egg. It is very difficult to remove entire eggs from the ovary at an early stage of their development, but during their later stages they may be removed entire if caution is taken. In this case they are not worked out in Mundie's macerating fluid but in the water from the aquarium. A series of measurements of the eggs and their nuclei is given below in Table I. These measurements were

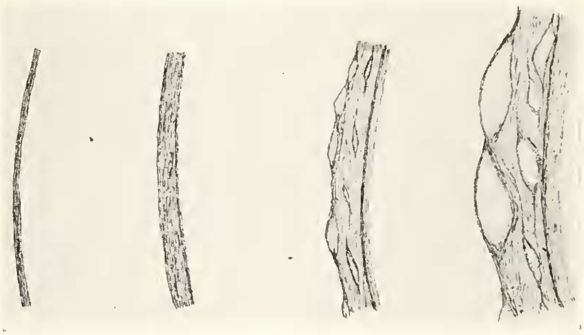
TABLE I

	Egg Diameter *microns*	Nuclear Diameter *microns*
1	1022.17	104.25
2	1035.50	103.5
3	1043.50	101.35
4	1124.2	115.45
5	1145.26	116.49
6	1178.47	116.78
7	1190.55	112.46
8	1232.48	134.19
9	1374.36	146.23
10	1413.57	156.42
Av. diameter egg	1176.004 Av. diameter nucleus	121.712

made on eggs and nuclei just before the process of maturation. We see from this table then that the average relation of diameter size is 1 to 9.66 + for the egg just before the process of maturation took place. Whether this nucleo-cytoplasmic relation has been maintained through the various periods of development is rather difficult to say, but it is interesting to note that several authors have observed that some eggs in their earlier stages are possessed of several nuclei, only one of which survives. Would this indicate then that this cell, which is to be the future egg, can increase in size only in relation to one nucleus or to only a given amount of nuclear material? If we would describe briefly the various stages of development from indifferent cell to the egg as it awaits fertilization, we should see that we have:

1. Indifferent cell (deriving liquid material from endoderm).
2. Very much enlarged indifferent cell (deriving liquid material from endoderm).
3. Very actively amœboid young egg (deriving solid and liquid material from endoderm and ectodermal ovarian cells).
4. The egg ceases amœboid activity and rounds up; nucleus lies near center of egg mass.
5. Migration of nucleus to periphery of egg.
6. Formation of first and second polar bodies with a consequent reduction of nuclear size to about one-eighth of its size at Stage 4.

We see then that this nucleus, up to Stage 4, is controlling a certain amount of cytoplasm; or vice versa, that the cytoplasm is increasing in amount in relation to a certain proportion of nuclear material. It appears that the cessation of the taking in of more material would indicate that after the nucleus has reached a certain size it controls the action of the cytoplasm, since it has been observed many times that when this amœboid activity ceases and the rounding-up process takes place there are many pseudocells very opportunely placed so that the cytoplasm of the egg could take them in with, may I be permitted to say, very little effort. Would this not indicate that the nucleus is through with its growth, since it allows no more additions to the cytoplasm, and that, at least at this period, it has a definite nucleo-cytoplasmic relation? This growth (to a certain size in relation to the cytoplasm) having been reached, the nucleus goes into the process of maturation during which the nuclear material is reduced. During this process the cytoplasm remains comparatively dormant and does not take any part



Text Fig. Nuclear membrane from normal to maturation stage. × 847.

in the cell's activity until the nucleo-cytoplasmic relation has been restored by the entrance of the sperm, which again restores the relation that previously existed.

The Nature of the Growing Nucleus

By the methods employed in this study, one is unable to see the small dark ovoid body near the nucleus or to measure the relation of the nucleus to the cytoplasm with any accuracy except in the later stages. The nucleus of the young egg, however, can always be distinguished from the nuclei of the surrounding enlarged interstitial cells. This distinction lies in its different refraction of the light and its more solid appearance. The nucleoli vary greatly in number during the growth of the nucleus and are apparently produced by the fusion of the many small nucleoli which are constantly being formed. The larger nucleolus apparently grows also in proportion to a certain nuclear size. One is constantly impressed, while observing these nuclei, that as far as appearance is concerned from the earliest stages up to the last stages no special change takes place except that of enlargement. The nuclear membrane, which seems to be thicker and more durable than that of other indifferent cells, is quite evident during the entire process of growth. The nodal points of the net enlarge; this growth seems to move inward toward the center of the nucleus during the entire process of growth. At the period of cessation of growth these enlarged nodal points give to the nucleus the appearance of being filled with thousands of large granules all very tightly packed together. The nuclear membrane at this stage is very tough and thin, and one soon learns to distinguish the nuclei which have just completed their growth by this characteristic. Later, this membrane is the first part of the nucleus to show evidences of change incident to the earlier stages of maturation as it increases in thickness and takes on a rather spongy appearance (see text figure). The nuclei which are in the earlier stages of maturation and display membranes such as the above were observed to shrink in size and eventually to become very much wrinkled over their surfaces as if the contained liquids of the nucleus were passing through this membrane into the surrounding medium. Nuclei which displayed the thin tough membranes were observed to maintain the same size over long periods of time, one having been kept for a period of thirty-eight hours.

Summary

1. The nucleus apparently maintains a relation of 1 to 9.66 to the cytoplasm in the development of the eggs of *Chlorohydra viridissimi*.

2. It appears that when the nucleus has attained a certain size no more cytoplasm is added to the egg.

3. During the process of growth of the nucleus the nodal points of the net increase greatly in size.

4. The number of nucleoli increases in relation to the size of the nucleus.

5. The nuclear membrane becomes highly vacuolated just prior to the process of maturation.

6. During the process of maturation the nuclear volume of the egg is reduced to about one-eighth of its volume prior to maturation.

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